FORMALIN AS A HARDENING AGENT FOR NERVE TISSUES.

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Formol, Formalin, or Formaldehyde was discovered by A. W. Hoffman in 1863 while passing wood spirit and air over a red-hot platinum spiral. If the vapor is brought into water to its point of saturation, a forty per cent. solution of formaldehyde is obtained, which has long been known under the name of formol. That Formalin possessed antiseptic as well as hardening powers we owe to the investigations of Dr. F. Blum, and these facts induced the elder Blum to make an extended series of investigations in the hardening of animal and vegetable tissues in the Senckenbergischen Institute at Frankfort, Germany. His preliminary report was published in the Zoölogischer Anzeiger, 1893, No. 434, and a more detailed report in the Berichte über die Senckenbergische Naturforscher Gesellschaft, 1894, p. 195. Here he details his experience, which in brief is as follows: Several human embryos were finely preserved in formol diluted with ten to twenty parts of water. Small embryos with amnion intact were preserved and the amniotic fluid remained transparent, so that the structural parts of the fœtus and the umbilical cord were recognizable. The mouse, hamster, and porpoise, were nicely preserved, the hair firmly in place and the eyes in better condition than under the use of alcohol. Reptiles, fishes and amphibians were nicely hardened in one to ten, one to twenty, or one to thirty, solutions according to the size of the object. The fishes retained in great part their color, while the slime and mucus covering them was rendered transparent. Of the invertebrates, snails, jelly fishes, insects, spiders, etc., all were well preserved.

Of the various animal tissues, muscles and the brain were quickly hardened, retaining the coloring matter of the blood in

the muscles, while in the brain the differentiation between the white and gray matter was very evident. Fruits, flowers and vegetables of various kinds were equally successfully preserved, the coloring matter very little if at all impaired. Blum's conclusions regarding the hardening of animal tissues may be summed up as follows :

Animal objects are hardened with shrinking, and without losing their microscopic structure or staining properties.

The natural form and color are preserved.

The eye remains much clearer than in alcohol.

The mucus of slime-producing animals is not coagulated and remains transparent.

The coloring matter of blood in tissues apparently disappeared, but may be quickly restored by a high per cent. alcohol.

These experiments of Blum were pathmaking and were quickly followed by those of Born (1), Pintner (2), Krückmann (3), Kenyon, Sadebeck, Mayers, and others with seemingly favorable results as regards the preserving and hardening powers of this compound. Besides these qualities it was especially valuable because of its being non-poisonous, non-combustible, of a low freezing point, and, what to scientists is quite a serious question, very cheap.

My attention was directed to this substance about one year ago, through experiments made at the Laboratory of the Erie County Hospital by my interne Dr. Helvie.

Various tissues, as liver, spleen, placenta, lungs, heart, muscles, and other tissues and organs were satisfactorily hardened and preserved. Especially gratifying were the results obtained with the umbilical cord, and other myxomatous tissues. Instead of shrivelling up and becoming opaque as occurs when alcohol is employed, the cord retained its normal size, was transparent and hardened to such a degree that sections were easily and perfectly

1. Med. Sect. d. Schlesisch. Gesellsch. f. Vaterl. Kultur. 1894.
2. Ver. Zool Gesell., Wien, 1894, p. 8.
3. Centralbl. f. Bakteriol und Parasitenk., 1894, pp. 851–57.
4. American Naturalist, Jan. 1, 1894.

cut with the microtome. The intestines were greatly shrivelled during hardening, but otherwise were a success.

These results induced me to try its virtues upon the brain and spinal cord and especially to find the earliest time when a spinal cord so hardened could be imbedded in celloidin and sections cut for staining and mounting. A spinal cord which to all appearance was normal was cut in pieces about one centimeter long and placed, sections of the cervical, thoracic and lumbar regions in bottles containing a five per cent. solution, ten per cent., twenty per cent. and twenty-five per cent. of Formalin. At the end of seven days a section of the cord was taken from each of these solutions and imbedded in celloidin, then placed on the microtome. The cord was evidently too imperfectly hardened as no good cuts were obtained. At the end of fourteen days the same procedure was followed, likewise on the twenty-first day and twenty-eighth day. From each of these intervals excellent cuts were obtained, the cord retained its external contour and appearance, but the differentiation between the white and the gray matter was not as well marked as when alcohol is used. These sections took the carmine stains nicely, but less so the nigrosin, Pal and Weigert stains. The most serious action of the formalin on all of these sections was a contraction, evidently of the neuroglia in various regions of the cord, especially of the white matter resulting in the formation of open spaces or cavities. In the sections hardened in the 10 per cent. solution these cavities were so large as to destroy completely the slides for microscopical purposes. In the 15 per cent. cord, the cavities were much smaller, but far more numerous, and the dorsal white columns looked like a honey comb or sieve. This action of the formalin was manifested in every section examined and is therefore not of accidental, but of regular occurrence. The drawback of the formalin in preventing the employment of the Pal and Weigert methods of staining has been successfully overcome by Marcus (1), who after hardening the cord for from two to four weeks in a one half per-cent. solution

1. Neurologisches Centralblatt, Jan. 1, 1895.

of Formalin, places small portions one-half cm. thick in Müller's fluid in a brood oven for seven days at a temperature of 37° C. They are then dehydrated, imbedded and the cuts again placed in Müller's fluid, for from two to seven days in a brood oven, quickly washed in alcohol, then transferred into the Weigert stain.

The action of the formalin on the ganglion cells is a happy one, swelling them and rendering their nuclei susceptible to very intense staining.

The action of formalin on the brain has given very fine results. Born succeeded in hardening the entire brain very quickly for demonstrations, also small particles for microscopical purposes. I have been equally successful and have some excellent specimens, nicely hardened. I have not as yet tried any of the staining methods on brains thus hardened and cannot state what the results would be, although I have some specimens under way. From my experience with Formalin I can greatly recommend it, for the hardening of the various organs and tissues for macroscopic as well as microscopic purposes, but would still cling to the Müller's solution for hardening the spinal cord, even if the time required for hardening be much longer than when Formalin is used.